

Normalizing Normal Mode Analysis

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A detailed understanding of protein dynamics remains elusive. Recent comparisons of computer simulations with experimental data are calibrating the methods and establishing their applications. In this issue of *Structure*, Kondrashov et al. (2007) exemplify the approach by comparing computed normal modes with measured crystallographic B factors.

"...everything that living things do can be understood in terms of the jiggings and wiggings of atoms."

From the Feynman Lectures in Physics (1970).

Few would disagree, but a generation later, the statement remains a prophecy rather than fact. Although 41,000 protein structures are known, there are none for which there is a complete description of the dynamics that is often key to understanding functional mechanism.

Despite keen interest in protein dynamics, studies have remained the purview of computer simulation while the challenges of experimental characterization are only slowly being overcome. Methods, such as molecular dynamics, are based on sound chemical physics but require approximations for application to large biomolecular systems (Karplus and McCammon, 2002). With each new methodology, a tension has emerged between the desire to characterize uncharted systems and the fear of the unknown impact of inherent assumptions and approximations on the simulations' results. A firmer footing can be established by comparing computed and experimental results when possible (Karplus and McCammon, 2002).

Until recently, experimental validations of normal mode calculations were largely qualitative. Since its introduction to biomolecules in the early 1980s, normal mode analysis (NMA) has allowed reduction in the dimensionality of simulations, and their extension to larger motions and longer timescales than hitherto accessible

(Ma, 2005). NMA's simplifying assumptions are that, as a structure is perturbed from equilibrium, the energy increase is parabolic, and that atoms move within the structure as coupled harmonic oscillators. Solutions of the Hamiltonian (energy) equation yield normal mode vectors describing the directions of collective atomic motions, their amplitudes and frequencies—at least in principle. The molecule's dynamics can then be approximated by a sum of normal mode vibrations. Excitement was heightened with the finding that the directions of known conformational changes often correlate with the sum of a few dominant modes, suggesting that NMA might be predictive of not just small oscillations, but of functionally relevant conformational dynamics (Figure 1).

NMA's application to large biomolecular systems requires crude approximations. Chief among these are elastic network model (ENM), and coarse-grained approximations that group several atoms into a single oscillator (Ma, 2005). In dynamics simulations, atoms are moved by forces calculated from the distortion of local stereochemistry. In ENM simplifications, the dependence of these forces on the types of atoms and chemical bonding is mostly ignored. With uncertainty as to the impact of the approximations, interpretations of NMA results have been largely qualitative, focusing on relative amplitudes and directions, but not frequencies. Three recent publications start to address these concerns through comparison of NMA to experimental B factors of known structures (Eyal et al., 2006; Hamacher and McCammon, 2006;

Kondrashov et al., 2007). B factors or thermal factors are a crystallographic measure of both dynamics and static disorder. Their scalar values are proportional to the mean square displacements of atoms from their mean positions. Surprisingly good correlations have been found between the experimental and NMA-derived magnitudes of disorder within each structure, and they have allowed calibration of the estimated forces between atom groups used in NMA calculations.

Compatible estimates of disorder are a gratifying validation of both approaches, but it is the directionality of normal modes that can provide a picture of functionally relevant conformational changes. Kondrashov et al. (2007) take comparison of NMA with crystallography one step farther. The authors focus on 83 proteins refined at high enough resolution to obtain anisotropic displacement parameters (ADPs), which, oversimplifying, can be considered to be thermal ellipsoids showing both the magnitude and the directionality of disorder. It was found that NMA methods, invoking different levels of approximation, differed in their agreement with experimental measurements. The best computer methods yielded motions within 50° of the crystallographically determined preferred directions, on average. The solid correlation observed between computation and experiment raises confidence that both measure real molecular dynamics/disorder. Moreover, the comparison provides an objective assessment of the alternative approximations used in NMA, charting a path toward more robust NMA calculations in the future.

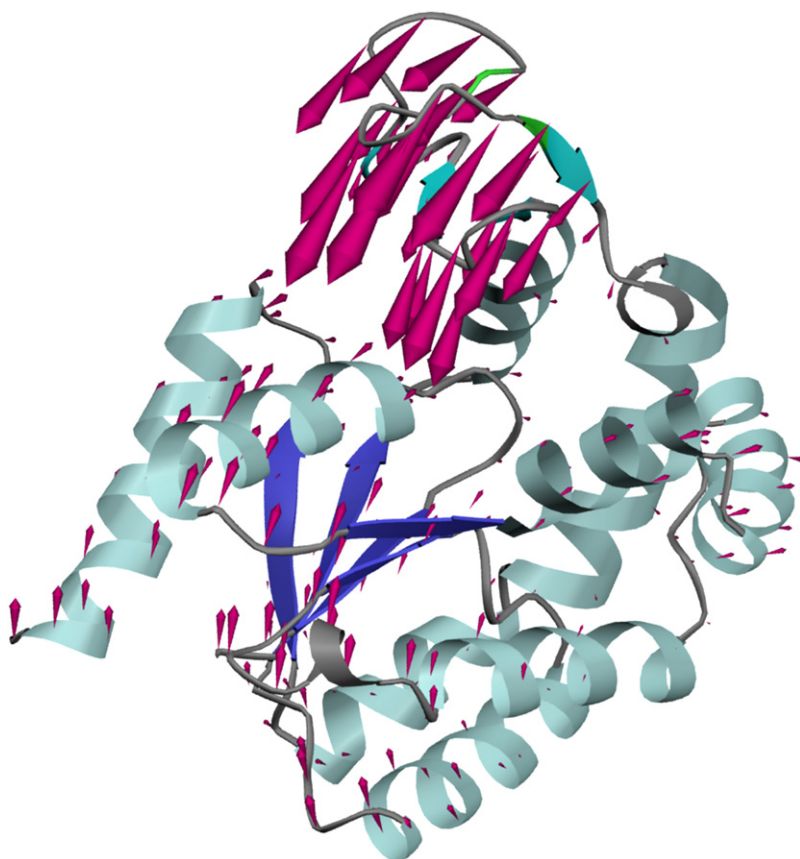


Figure 1. Normal Mode Vibrations Can Be Predictive of Large-Scale Conformational Changes

Adenylate kinase has been the focus of many normal mode analyses. Calculations of the protein's intrinsic dynamics have captured the directions of motion seen crystallographically in substrate-induced domain motions (Gerstein et al., 1993). An analysis with the program ElNemo (Suhre and Sanejouand, 2004) illustrated here shows arrows proportional to the amplitudes of the lowest frequency normal mode. Comparisons of such calculations against crystallographic B factors (Kondrashov et al., 2007) will increase the confidence with which functional dynamics can be dissected. (Figure provided by R. Bannen and G. Phillips, Jr., University of Wisconsin.)

There are many incompatibilities between NMA and crystallography that make the observed correlations remarkable. On the NMA side, there are the approximations of coarse-graining, elastic network models, harmonic assumptions, and lack of solvent. On the crystallographic side, thermal factors are a harmonic modeling of partially anharmonic disorder, reflect a crystal lattice environment, incorporate static conformational variability, and, in the current high-resolution studies, reflect dynamics below the glass transition temperature. Finally, B factor theory unrealistically assumes incoherent/independent atomic oscil-

lations, in contrast to the coupled oscillations of NMA.

The recent publications comparing theory and experiment in protein dynamics are path-breaking, but likely not the final word. It may be possible to reduce the incompatibilities between current experiments and calculations by explicit accounting for temperature, lattice environment, and so forth. Closer correspondence would allow improved calibration of NMA calculations. One should also note that both NMA and B factor theory assume harmonic perturbations from an equilibrium structure. The compatibility of the current estimates may stem from

both methods' sampling of mostly fast oscillations of modest amplitude. Perhaps the greatest excitement is over application of NMA to large enzymes and macromolecular machines, which requires extrapolation of the methodology to much larger, slower, and anharmonic motions. A few ADP and NMA analyses have indicated that nature sometimes takes advantage of soft or easy conformational fluctuations to forge a path to larger conformational changes (e.g., Hinsen et al., 1999; Yousef et al., 2002). However, calibration of the computational methods for quantitative interpretations of such systems will require experimental measures of nonharmonic dynamics in the functionally relevant micro- to millisecond time regimes. For this future work, we will have to look beyond crystallography and conventional NMR order parameter analysis (Kay, 1998), perhaps to the NMR measures of relaxation dispersion or residual dipolar coupling that are being developed (Blackledge, 2005; Cavanagh and Venters, 2001).

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